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Liquid Biopsy v Tissue NGS testing in Oncology: Competing Option or a Complementary Strategy?

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Disclosures

- **Dr Gerald Martin**
- Employed by OncoDNA as Consultant
- No other affiliations

- **Dr Petra Pokorná**
- Employed by CEITEC Masaryk University and University Hospital Brno as Research and Laboratory Methods Specialist
- No other affiliations

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What You Will Gain From This Presentation

- Learn about the speakers!
- A brief overview of principles behind liquid biopsies
- Some limitations and benefits of liquid biopsy v tissue testing.
- Which ctDNA liquid biopsy technologies available to clinicians
- Insight from a multi-center validation of the OncoSELECT ctDNA assay

- The usefulness of liquid biopsy testing for clinicians
- An examination of where liquid biopsy testing fits into the clinic?
- Challenges of validation and availability of suitable samples
- Challenges of funding for liquid biopsy
- Future prospects for liquid biopsy testing in the clinic - What hurdles/issues still need to be overcome?
- A summary of key points
- An opportunity to ask questions

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Introductions

- **Dr Gerald Martin**
- BA(Hons) and PhD at Dublin University (Trinity College Dublin)
- Post-docs at Beatson Institute, Glasgow & Cambridge University, UK.
- Held technical and commercial roles at several companies for more than 25 years, focusing on clinical NGS for the last 12 years.
- Most recently held FAS/SME roles at Oxford Gene Technologies, Sophia Genetics and Velsera (PierianDx).
- Joined OncoDNA as Commercial Applications Scientist in October 2024.

- **Dr Petra Pokorná**
- BSc, Masters (Medical Genetics and Molecular Diagnostics) and PhD candidate (Biochemistry) at Masaryk University, Brno.
- Joined Central European Institute of Technology (CEITEC) as Research Scientist in 2019.
- Has also held role of Research and Development Specialist at University Hospital, Brno since 2021 where Petra investigates and implements new diagnostic technologies for routine clinical use.

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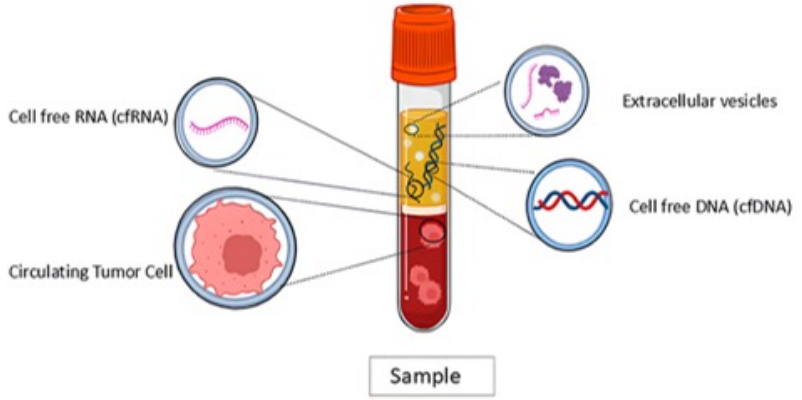
Liquid Biopsy Principles

- Liquid biopsy assay - any test that identifies clinically-relevant biomarkers from a biofluid (eg blood, cerebrospinal fluid (CSF) or urine).
- Analytes could be tumor cells, nucleosomes, nucleic acids, proteins or epigenomic markers (- here we will focus on nucleic acids).
- There are multiple assay types (- here we will focus on NGS).

- As cancer cells die, circulating tumor DNA (ctDNA) can be released into the blood stream (or other biofluids).
- This can be targeted by the liquid biopsy assay.
- Normal cells can also release DNA as they die
  - ctDNA is a subset of all cell-free DNA (cfDNA) in the biofluid.



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Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Why use liquid biopsy assays?

- Solid tumor tissue may be inaccessible or dangerous to biopsy
  - Tissue sampling may put patient at risk of infection, excessive bleeding or the risk of puncturing nearby organs (eg lung)
- Tissue sample material can be diffuse or sparse (eg lung)
  - Can enough cancer DNA be extracted for necessary testing?
- Tissue sampling is invasive and difficult to perform
  - Tissue biopsies are more specialized and may require special equipment or skills
  - Tissue biopsies are generally unpleasant for the patient and may be difficult to repeat
- Solid tumors evolve - tissue samples may not reflect the most active part of the tumor mass
  - Tumor evolution or heterogeneity may confound correct treatment assessment
- Tissue samples are slower and more complicated to process
  - Time can be lost before the correct treatment is started

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Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Why use liquid biopsy assays?

- Liquid biopsies are more readily accessible
  - A simple blood draw may suffice
- Liquid biopsy are in relatively large supply
  - Ample material can be taken (in theory) and sampling can be repeated easily
- Liquid biopsy sampling is much less invasive and easier to perform
  - No special equipment, theatre time or skills required
  - Much less unpleasant for the patient
- Liquid biopsies are more dynamic and reflective of the tumor as a whole
  - Tumor evolution and heterogeneity should be captured, not missed
  - Current tumor activity (rather than a historical view) is monitored
  - There is a prognostic correlation between higher ctDNA levels and poor outcomes
- Liquid biopsies can be processed more rapidly in the lab
  - Time to report can be minimized – which could expedite treatment initiation

Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Liquid biopsy assays? What are the 'Cons'?

- Uncertainty about ctDNA shedding
  - Not all cancer types shed ctDNA. What does a negative result mean?
- Lower limit of detection is required
  - Remission MRD testing requires much lower limit of detection than tissue testing
- Validation may be challenging due to limited availability of controls
  - Samples for validation should have been tested directly by another assay
- Clonal haematopoiesis can complicate analysis
- More uncertainty about ctDNA shedding…
  - An elephant in the room is that stage 1 cancers that are buried deep in the tissue, are unlikely to shed ctDNA into the blood – so early screening may be less likely to be fruitful
- Cost (due to panel and sequencing depth requirements)
  - Sequencing depth can be as high as 30000 x

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Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Liquid biopsy assays? What are the 'Cons'?

- Uncertainty about ctDNA shedding
  - Not all cancer types shed ctDNA. ctDNA half-life. What does a negative result mean?
- High and Low shedding cancers
  - **Higher ctDNA levels are generally associated with larger or more aggressive tumors**
  - **But certain tumor types release more ctDNA than others –**
  - advanced stages of ovarian, liver, pancreas, bladder, colon, **lung (squamous tumors more than adenocarcinomas)**, stomach, **breast (triple-negative breast cancer more than other types)**, liver, esophagus, and head and neck cancers as well as neuroblastomas and melanomas
  - **Tumors containing *TP53* variants or Copy Number gains seem to shed more ctDNA**
  - **Lower levels of ctDNA are associated with other tumors –**
  - ctDNA detection is **challenging** in **medulloblastomas**, or **kidney**, **prostate** or **thyroid cancer**.
  - **CNS tumors** shed less ctDNA into the blood (eg the blood of 90% of glioma patients do not harbor detectable levels of ctDNA) – CSF liquid biopsy may be better?

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Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Liquid biopsy assays? What are the 'Cons'?

- Lower limit of detection is required
  - Remission MRD testing requires much lower limit of detection than tissue testing
  - Not as technically demanding as in the past
  - Bioinformatic analysis must be refined to avoid false positives

- May be challenging to validate due to limited availability of controls
  - Samples for validation should have been tested directly by another assay
  - Previously-tested clinical samples are limited - Will improve with collaborations
  - How do you establish 'gold standard' results? ddPCR?
    - Important to establish reliable Variant Fraction determination
    - Other biomarkers – MSI, TMB, HRD, CNVs – copy gains, copy losses

Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Liquid biopsy assays? What are the 'Cons'?

- Clonal haematopoiesis can complicate analysis
  - Variant fraction alone is probably not enough to distinguish CHIP as tumor-derived variants often have low VAF, but…
  - May not be as big an issue for solid tumors as first appears - Relevant for older patients only (~5% of solid tumors?)
  - Typically involves selected genes – *DMNT3A*, *TET2*, *ASXL1*, *TP53*, *JAK3*, *SF3B1*, *GNB1*, *CBL*, *SRSF2* and *GNAS*
  - CHIP variants are not directly relevant for targeted cancer therapy
  - The cancer type will already be known, so the relevance of variants in these genes can be downscaled in that context
  - Strategies for paired ctDNA-(white cell) gDNA NGS testing – Recommended by ESMO (but if paired WBC sequencing not possible, "conservative reporting should be applied")
  - Paired sequencing is costly – should both samples be tested at the same time or better to perform gDNA testing on potentially positive samples only?
  - Could smaller gDNA panel be used instead of the same large panel?

Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Liquid biopsy assays? What are the 'Cons'?

- More uncertainty about ctDNA shedding…
  - Could liquid biopsy be used for "pre-symptomatic" cancer screening?
  - Cost is certainly an issue – sequencing thousands of samples to identify a few potential solid tumor cancers is still a long way off
    - Is there an outcome and/or cost benefit to very early detection? (Will these patients relapse anyway
  - Is it even possible?
    - Are early cancers shedding ctDNA? Are available assays sensitive enough?
    - Only 42% of Stage I NSCLC showed detectable ctDNA (v 67% and 88% Stage II, III)
    - 50% of localized NSCLC shed ctDNA at less than 0.01% of the cfDNA
  - No significant improvement over current population screening programs has been proven yet –
    - (GRAIL Galleri, PATHFINDER 2 disappointing? GRAIL-NHS end point missed? More data in 2026?)

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Limitations And Benefits Of Liquid Biopsy v Tissue Testing

Liquid biopsy assays? What are the 'Cons'?

- Cost (due to panel and sequencing depth requirements)
  - Sequencing depth can be as high as 30000 x
  - With larger sequencing platforms, new technologies and new providers – this is also not as challenging as in the past
  - Panel costs vary greatly. Even smaller panels can be expensive.
  - But where does funding come from? Who is paying?
    - Tissue still considered the gold standard and ESMO-recommended approach. What programs exist in your region to reimburse liquid biopsy testing?
- Cost v Value for money?
  - Smaller panels may be cheaper but are they better value?
  - What cancers are you testing? Are all relevant genes and guidelines covered?
  - Time? Wet-lab and dry-lab considerations – Analysis and reporting
  - Time? QC and repeat assays?

ctDNA Liquid Biopsy Technologies Available To Clinicians

Multiple ctDNA assays are available commercially as services or kits -

Services:

- **OncoDNA (OncoSELECT)**
- Guardant Health
- CERBA
- CeGaT
- Genekor
- Natera (Signatera)
- Sentis Discovery
- Gene Solutions
- Novogene
- Tempus
- Neogenomics
- Labcorp
- Personalis
- Caris Life Sciences
- Foundation Liquid
- Synnovis
- Synlab
- GRAIL
- ALMAC
- IOZK
- Genekor
- Gene+
- Inocras
- Exact Sciences
- Burning Rock
- Lucence
- Cancer Cell Diagnostics

Kits:

- **OncoDNA (OncoSELECT, OncoXPLORE, OncoFOLLOW)**
- Illumina
- Thermo
- Qiagen
- Hedera
- AmoyDx
- PGDx
- 4baseCare
- Burning Rock
- genes2me (G2M)
- MSK Impact / Sophia Genetics
- Twist
- Agilent
- IDT / ArcherDx
- Roche
- Pillar BioScience
- Sysmex
- Nonacus
- Celemics
- Gene+

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ctDNA Liquid Biopsy Technologies Available To Clinicians

- Lots of choice
- The question is not “what is best”, but “what is most appropriate for me”
- What do you want to achieve?
- What are your applications and constraints?

- Rapid treatment guidance?
- Profiling of cancers that are difficult to assess with tissue?
- Treatment response and prognosis evaluation?
- Remission **MRD** (or early relapse detection)?
  - OncoSELECT > OncoXPLORE > OncoFOLLOW
- Relapse/resistance profiling?
- Treatment guidance or biomarker research?
- Population screening?
  - Efficacy v cost v benefit

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ctDNA Liquid Biopsy Technologies Available To Clinicians

- Lots of choice
- The question is not “what is best”, but “what is most appropriate for me”

- Are you looking for a cancer-specific panel or a broad profiling panel?
- Are the genes of your particular interest in the panel?
- Is MRD important or is treatment guidance the goal?
- Do you need the flexibility of a parallel kit and service offering?
- Is price the over-riding concern?
- Are you constrained by sample numbers?
- Do you need to multiplex multiple tests in the same sequencing run?
- Is sequencing capacity a constraint?
- Is automation (across multiple assays) important?

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ctDNA Liquid Biopsy Technologies Available To Clinicians

Provider	Test	Genes	Cancers	Sarcomas	LOD VF	Sensitivity	Cost	Tumor:Normal	Pre-Screening	Therapies	Prognosis	MRD	Relapse	Resistance	Kit and Service	Routine	Research	Whole gene	SNVs	Indels	CNVs	Fusions	Fragmentomics	Methylation	TMB	MSI
OncoDNA	OncoSELECT	74	Pan	No	0.25%	>99%	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	No	No
OncoDNA	OncoFOLLOW	WES/<100	Pan	Yes	0.003%	>99%	High	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	Yes	No
OncoDNA	OncoXPLORE	443	Pan	Yes	0.25%	>95%	Moderate	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Guardant Health	Guardant 360	744	Pan	?	0.20%	>99%	High	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Mostly	Yes	Yes	Limited	Limited	Yes	Yes	Yes	Yes
Guardant Health	Reveal MRD		Limited (3)	Np	0.01%	?	High	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No	No	No	No	Yes	No	No
CeGaT	CancerDetect	32	Pan	No	0.20%		Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Yes	No	Hotspot	Yes	Yes	No	No	No	No	No	No
CeGaT	CancerPrecision	>700	Pan	No	5%	>95%	High	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes
CeGaT	CancerMRD	WGS/<50	Pan	Yes	0.1%	?	High	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Mostly	Yes	Yes	Yes	No	No	No	Yes	Yes
Sentis Discovery	Cancer + Discovery (ctDNA)	688/816	Pan	?	?	?	Moderate	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Mostly	Yes	Yes	Limited	Limited	No	No	Yes	Yes
Gene Solutions	K-4Care	>700	Pan	?	?	?	High	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Gene Solutions	K-Track	155	Pan	?	?	?	High	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Mostly	Yes	Yes	Yes	No	Yes	No	No	No
Foundation	Foundation One Liquid CDx	324	Pan	No	?	?	High	No	No	Yes	Yes	No	Yes	Yes	Yes*	Yes	Yes	Yes	Yes	Yes	Yes	Limited	No	No	Yes	Yes
GRAIL	Galleri		Pan (50)	Yes	?	PPV 62%	High	No	Yes?	No	Yes	No	Yes	Yes	No	Yes	Yes		No	No	No	No	No	No	No	No
Natera	Signatera	WES/<100	Pan	Yes	?	?	High	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	No	No	No	No	No
Tempus	xM	523	Pan	No	0.50%	>95%	High	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Neogenomics	NEO PanTracer LBx	517	Pan	No	0.17%	>96%	?	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Limited	No	No	Yes	Yes
Labcorp	Plasma Complete	521	Pan	No	0.500%	>99%	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Limited	No	No	Yes	Yes
Labcorp	Plasma Detect	WGS/5000	Pan	No	0.005%	>99%	High	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Limited	No	No	No	No
Personalis		WGS/?	Pan	Yes	0.001%	?	High	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	?	?	No	No	No	No
Inocras	MRDVision	WGS	Pan	Yes	0.001%	>94%	High	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	?	?	No	No	No	No
Inocras	CancerVision	WGS/600	Pan	Yes	0.5%	>99%	High	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Cancer Cell Diagnostics	CellSight	>500	Pan	No	5.0%	>99%	High	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Novogene	NovoPM 2.0	484	Pan	?	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Synnovis	(MSK)	147	Pan	No	0.50%	>95%	High	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	No	No
CERBA	Cerba OncoSign ctDNA	42 (17 RNA)	Limited	No	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	?	?	No	No	No	No
CERBA	(TSO500)	523	Pan	No	0.50%	>95%	High	No	No	Yes	Yes	No	Yes	Yes	No*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
ALMAC	(TSO500)	523	Pan	No	0.50%	>95%	High	No	No	Yes	Yes	No	Yes	Yes	No*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
IOZK	*liquid*biopsy	74	Pan	No	0.25%	>99%	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	No	No
Genekor	(Gene-)	1020	Pan	?	0.20%	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Gene+	(Gene-)	1020	Pan	?	0.20%	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Caris Life Sciences	Caris Assure	WES	Pan	?	0.50%	?	High	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Exact Sciences	Oncodetect	WES/200	Pan	?	0.0015%	?	High	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	No	No	No	No	No
Burning Rock	OncoCompass	520	Pan	?	2%	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes*	Yes	Yes	Yes	Yes	Yes	Limited	Limited	No	No	Yes	Yes
Burning Rock	OncoCompass	101	Pan	?	0.40%	>98%	Moderate	No	No	Yes	Yes	Yes	Yes	Yes	Yes*	Yes	No	Yes	Yes	Yes	Limited	No	No	No	No	No
Burning Rock	CanCatch Custom	Custom	Pan	?	0.40%	>98%	High	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	?	?	No	No	No	No
Burning Rock	OncoC Multi Cancer		Limited	No	?	>69%	?	No	Yes?	Yes	Yes	Yes	Yes	Yes	Yes*	Yes	No	No	No	No	No	No	No	Yes	No	No
Lucence	LiquidHallmark	79	Limited	No	0.10%	>96%	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes*	Yes	No	No	Yes	Yes	No	Yes	No	Yes	No	No

ctDNA Liquid Biopsy Services

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ctDNA Liquid Biopsy Technologies Available To Clinicians

Provider	Test	Genes	Cancers	Sarcomas	LOD VF	Sensitivity	Cost	Tumor:Normal	Pre-Screening	Therapies	Prognosis	MRD	Relapse	Resistance	Kit and Service	Sample Nos	Multiplexing	Agnostic	Automation	Seq Capacity	Routine	Research	Whole gene	SNVs	Indels	CNVs	Fusions	Fragmentomics	Methylation	TMB	MSI
OncoDNA	OncoSELECT	74	Pan	No	0.25%	>99%	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	8	Yes	Yes	Yes	Moderate	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	No	No
OncoDNA	OncoFOLLOW	WES/<100	Pan	Yes	0.003%	>99%	High	No	No	Yes	Yes	Yes	Yes	Yes	Yes	2	Yes	Yes	Yes	High	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	Yes	No
OncoDNA	OncoXPLORE	443	Pan	Yes	0.25%	>95%	Moderate	No	No	Yes	Yes	Yes	Yes	Yes	Yes	8	Yes	Yes	Yes	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Illumina	TSO500	523	Pan	No	0.50%	>95%	High	No	No	Yes	Yes	No	Yes	Yes	No*	8	Limited	No	Yes	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Thermo	OncoMine	52	Limited	No	0.10%	>80%	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Flexible	Limited	No	Yes	High	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	No	No
Qiagen	Ultra Human Actionable	95	Pan	No	0.10%	>99%	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Flexible	Limited	No	Yes	Moderate	Yes	Yes	Limited	Yes	Yes	Yes	No	No	No	No	No
Hedera	HP2	32	Limited	No	0.50%	>99%	Low	No	No	Yes	Yes	No	Yes	Yes	No	8	Limited	No	No	Low	Yes	No	Mostly	Yes	Yes	Yes	No	No	No	No	Yes
AmoyDx	various	152/24	Pan	No	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes*	8	Limited	No	No	Moderate	Yes	No	Mostly	Yes	Yes	Yes	Limited	No	No	?	?
PGDx	various	521/33	Pan	?	0.1%/0.4%	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	8	Limited	No	No	Moderate	Yes	No	Mostly	Yes	Yes	Yes	Limited	No	No	?	?
4baseCare	TARGT	1212	Pan	?	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	?	Limited	No	No	Moderate	Yes	No	?	Yes	Yes	Yes	Limited	No	No	?	Yes
4baseCare	TARGT	72	Pan	?	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	?	Limited	No	No	Moderate	Yes	No	?	Yes	Yes	?	No	No	No	No	No
Burning Rock	OncoCompass	520	Pan	?	2%	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes*	?	Limited	No	Limited	Moderate	Yes	Yes	Yes	Yes	Yes	Limited	Limited	No	No	Yes	Yes
Burning Rock	OncoCompass	101	Pan	?	0.40%	>98%	Moderate	No	No	Yes	Yes	Yes	Yes	Yes	Yes*	?	Limited	No	Limited	Moderate	Yes	No	Yes	Yes	Yes	Limited	No	No	No	No	No
Burning Rock	OncoC Multi Cancer	? (70K loci)	Limited	No	?	>69%	?	No	Yes?	Yes	Yes	Yes	Yes	Yes	Yes*	?	Limited	No	Limited	Moderate	Yes	No	No	No	No	No	No	No	Yes	No	No
genes2me (G2M)	CancerCheck 100	148	Pan	No	<1%?	>95%	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	8	Limited	Yes	Yes	Moderate	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes
genes2me (G2M)	Lung, Breast, Colorectal	48/37/35	Cancer specific	No	<1%?	>95%	Low	No	No	Yes	Yes	No	Yes	Yes	No	8	Limited	Yes	Yes	Low	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
Twist	Twist Oncology - DNA CGP	562	Pan	?			Moderate	No	No	Yes	Yes	No	Yes	Yes	No	8	Yes	Yes	Yes	Moderate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Agilent	SureSelect	Custom	Custom	Custom	0.10%	unclear	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	8	Limited	No	Limited	Low	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Agilent	Avida	680/105	Pan	Yes	0.20%	>94%	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	8	Limited	No	Limited	Moderate	Yes	Yes	Hotspots	Yes	Yes	Yes	No	No	No	No	No
IDT / ArcherDx	various	29	Limited	No	0.50%	>99%	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Flexible	Limited	No	Limited	Low	Yes	No	Hotspots	Yes	Yes	Yes	Yes	No	No	No	No
Roche	Avenio Surveillance (FM)	197	Pan	?	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes*	8	Limited	No	Limited	Moderate	Yes	Yes	Limited	Yes	Yes	Few	Yes	No	No	No	No
Roche	Avenio Expanded	77	Pan	?	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	8	Limited	Limited	Limited	Moderate	Yes	No	Limited	Yes	Limited	Limited	Limited	No	No	No	No
MSK Impact / Sophia Genetics	ACCESS	147	Pan	No	0.50%	>95%	High	Yes	No	Yes	Yes	No	Yes	Yes	Yes	8	Limited	No	Yes	High	Yes	Yes	Mostly	Yes	Yes	Yes	Yes	No	No	No	No
Pillar BioScience	Core	104/34	Pan	No	0.10%	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	Flexible	Limited	No	Limited	Moderate	Yes	No	Mostly	Yes	Yes	Yes	No	No	No	No	No
Sysmex	Custom/Leuk		Limited	No	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	No	8	Limited	No	Limited	Moderate	Yes	No	Mostly	Yes	Yes	Yes	No	No	No	No	No
Nonacus	GALEAS	519	Pan	No	?	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	8	Limited	No	Limited	Moderate	Yes	No	Mostly	Yes	Yes	Yes	No	No	No	No	No
Celemics	Lung, Breast, Colorectal	28/27/15	Cancer specific	No	0.50%	>94%/>99%	Moderate	No	No	Yes	Yes	No	Yes	Yes	No		Limited	No	Limited	Moderate	Yes	No	Limited/Yes/Yes	Yes	Yes	Yes	No	No	No	No	No
Gene+		1021	Pan	?	0.20%	?	Moderate	No	No	Yes	Yes	No	Yes	Yes	Yes	8	Limited	No	Limited	Moderate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Gene+		188	Pan	?	0.20%	?	High	No	No	Yes	Yes	Yes	Yes	Yes	Yes	8	Limited	No	Limited	Moderate	Yes	No	Limited	Yes	Yes	Yes	Yes	No	No	No	No

ctDNA Liquid Biopsy Kits

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When To Use Liquid Biopsy Testing?

Liquid biopsy and tissue assays in parallel? A complementary approach.

- When possible, a liquid first approach for fast results
  - Rapid testing with a liquid biopsy assay could help initiate treatment faster
  - Could a liquid biopsy sample be taken during the specialist's consultation prior to the hospital appointment (for solid tissue biopsy)?
  - Can liquid biopsy be used to guide scanning or tissue biopsy targeting?
- Follow-up appointment for tissue biopsy allowing comprehensive testing
  - Tissue testing still considered the 'Gold standard'
  - Confirming and complementing the initial liquid results
  - Ensure nothing relevant has been missed
- Liquid biopsy testing for prognostic treatment assessment and MRD
  - Liquid testing as remission starts and during remission (to detect resistance or relapse)
- Parallel testing at relapse
  - Rapid testing by liquid biopsy (with possible identification of rogue sites)
  - Complete profiling from metastatic tissue to confirm treatment options

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Overview Of Multi-Center OncoSELECT ctDNA Assay Validation

OncoSELECT by OncoDNA KIT

From tumor to treatment

A comprehensive oncology NGS panel with powerful bioinformatics tools for the latest clinical insights.

OncoSELECT Gene Panel

OncoSELECT is suited for solid cancer patients' testing, and covers many alterations associated with targeted/ hormonal therapies

Total : 74 Genes: SNV / Indels / CNV / TERT promotor / Intron aberrations *(gene translocations, unusual splicing) / MSI* ∆

28 genes : all exons

ARID1A	CHEK2	NBN
ATM	FANCA	NF1
ATR	FANCL	PALB2
BARD1	**FGFR1**	PTEN
BRCA1	**FGFR2**	RAD51C
BRCA2	**FGFR3**	RAD51D
BRIP1	H3C2	RAD54L
CDK12	KEAP1	STK11
CDKN2A	MLH1	
CHEK1	MRE11A	

37 genes : hotspots

AKT1	ESR1	**KIT**	PIK3CA
ALK	FOXL2	KRAS	POLE
AR	GNA11	MAP2K1	RAD51B
ARAF	GNAQ	**MET**	**RET**
BRAF	GNAS	MYOD1	**ROS1**
CTNNB1	H3F3A	NRAS	pTERT
DICER1	H3F3B	**NTRK1**	TP53
EGFR	HRAS	**NTRK2**	
ERBB2	IDH1	**NTRK3**	
ERBB4	IDH2	PDGFRA	

22 genes associated with translocations

ALK	KIF5B	SDC4
BRAF	**KIT**	SLC34A2
CD74	**MET**	
EGFR	NPM1	
EML4	NRG1	
ETV6	**NTRK1**	
EZR	**NTRK2**	
FGFR1	**NTRK3***	
FGFR2	**RET**	
FGFR3	**ROS1**	

3 unusual splicing genes

BRCA1

BRCA2

MET

*NTRK3 fusion is detected via its common rearrangement partner ETV6

Bold : Gene duplicated

∆ *Under validation*

OncoDNA GROUP

Overview Of Multi-Center OncoSELECT ctDNA Assay Validation

- Multiple sites performed testing
  - OncoDNA SA, Gosselies, Belgium;
  - Immun-Onkologisches Zentrum KÖLN (IOZK), Cologne, Germany;
  - Pathology Department, Hospital del Mar, Barcelona, Spain;
  - Institut de Biochimie et Biologie Moléculaire, Centre Hospitalier Universitiare de Lille, Lille, France
- Different Illumina sequencing platforms used (14 runs)
  - NextSeq 500, NextSeq 2000, NovaSeq 6000, NovaSeq X Plus
- Range of ctDNA extraction kits/protocols used
- Reference samples
  - Multiple reference samples used – some across all sites: Twist, SeraSeq,
- Clinical samples
  - 81 previously-tested clinical samples with confirmed variants
- Multiple Replicates included for repeatability and reproducibility

- Testing types of variants - SNVs, indels , fusions, CNVs

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Overview Of Multi-Center OncoSELECT ctDNA Assay Validation

	SNVs	Indels	Fusions		CNVs
			2 genes targeted	1 gene targeted	
Limit of Blank	0.114% VF	0.172% VF	0%	0%	0%
Limit of Detection	0.25% VF	0.25% VF	0.5%	1%	6 copies
Sensitivity	>96.52%	>95.83%	>97.6%	>99%	>99%
Specificity	>99%	>99%	>99%	>99%	>99%
Accuracy	>99%	>99%	>99%	>99%	>99%

- OncoSELECT – Available now as kits for 4 hybridizations (up to 32 samples)

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Liquid Biopsy v Tissue NGS testing in Oncology: Competing Option or a Complementary Strategy?

Dr Gerald Martin

Commercial Applications Scientist, OncoDNA

Dr Petra Pokorná

Specialist in Laboratory Methods, Center for Precision Medicine, University Hospital Brno, Czechia

The Usefulness Of Liquid Biopsy Testing For Clinicians

- **More accessible**
  - Minimally invasive sampling
  - Low procedural burden
- **More dynamic**
  - Rapid and repeatable sampling allowing longitudinal assessment
- **More representative**
  - Resolving spatial heterogeneity
  - Systemic signal in multi-lesional disease

➡ **Streamlined access to actionable genomic information**

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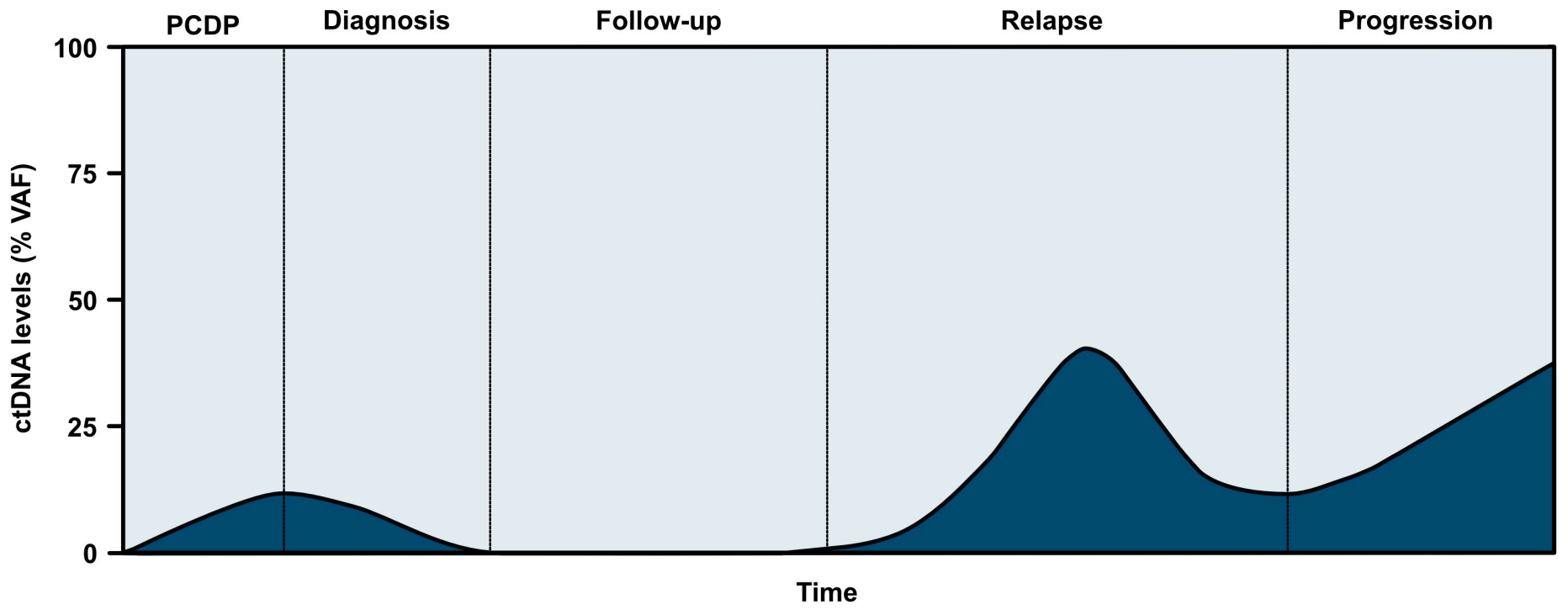
Where Does Liquid Biopsy Testing Fit Into The Clinic?



PCDP/Initial diagnosis

- Screening
- Baseline profiling

Longitudinal follow-up

- MRD detection
- Response monitoring
- Correlation with clinical evaluation/imaging

Relapse/progression

- Mechanisms of resistance
- Treatment selection
- Capturing heterogeneity in metastatic disease

Figure adapted based on Pascual et al., 2022, DOI: 10.1016/j.annonc.2022.05.520

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Challenges Of Validation And Availability Of Suitable Samples

Challenges related to suitable validation samples

- **Access to representative samples**
  Relevant diagnoses, disease stages, treatment settings
- **Paired clinical and molecular data**
  Disease stage, imaging results, tissue NGS
- **Appropriate control samples**
  Context-dependent selection of non-malignant comparators
- **Availability of follow-up samples**
  Less commonly biobanked
  Establishing clinically defined comparable timepointS

Intrinsic analytical and biological challenges

- **Standardized sample processing**
  Consistent and documented sample handling during pre-analytical steps, logistics
- **Low ctDNA abundance in specific scenarios**
  Limited tumor shedding in early-stage disease
- **Assay sensitivity threshold**
  Distinguishing true tumor-derived signals from biological and technical background noise
- **Biological background signals affecting specificity**
  Non-tumor-derived cfDNA contributions, including clonal hematopoiesis

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University Hospital Brno Pilot Testing Cohort

- No liquid biopsy biobanking at UHB – prospective enrollment in cooperation with our outpatient clinic for adult solid tumors
- 2 vials of peripheral blood collected into cfDNA collection tubes when patients consented to participate
- 11 adult patients with different diagnoses and different disease stages (10 selected and approached by an oncologist, 1 patient enrolled upon active interest in the testing)
- Control samples required for some NGS solutions – commercial controls (positive CTRL), healthy volunteers (negative CTRL)

University Hospital Brno Pilot Testing Cohort

Patient no.	Diagnosis	Disease course at the liquid biopsy collection	Tumor NGS data available	Collection timepoint of the available tissue biopsy	LB collection timepoint with respect to CGP indication
1	Adenocarcinoma of pancreas	Primary diagnosis, early stages of treatment	Yes	1-3 months prior to CGP	In parallel or <2 months
2	Cholangiocarcinoma of biliary tract	Primary diagnosis, early stages of treatment	Yes	1-3 months prior to CGP	In parallel or <2 months
3	Malignant tumor of unknown origin	Primary diagnosis, early stages of treatment	Yes	1-3 months prior to CGP	In parallel or <2 months
4	Adenocarcinoma of cecum	Progressive disease, on treatment	Yes	10 months prior to CGP	14 months after CGP during documented progression
5	Adenocarcinoma of sigmoid colon	Progressive disease, on treatment	Yes	10 months prior to CGP	5 months after CGP at follow up
6	Sarcoma	Primary diagnosis, early stages of treatment	Yes	1-3 months prior to CGP	In parallel or <2 months
7	Adenocarcinoma of pancreas	Progressive disease, on treatment	Yes	22 months prior to CGP	In parallel or <2 months
8	Adenocarcinoma of pancreas	Primary diagnosis, early stages of treatment	Yes	1-3 months prior to CGP	In parallel or <2 months
9	Triple-negative breast cancer	Progressive disease, on treatment	Yes	5 years prior to CGP	In parallel or <2 months
10	Intrahepatic bile duct carcinoma	Progressive disease, on treatment	Yes	1-3 months prior to CGP	17 months after CGP during documented progression
11	ALK-positive non-small cell lung carcinoma	Progressive disease, on treatment	No*	NA	NA

*testing done outside of UHB, basic molecular information provided (*ALK* rearrangement, *MET* amplification)

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Low ctDNA Abundance In Specific Scenarios

- Variable yield within diagnoses and within diagnostic groups

Patient no.	Diagnosis	Disease course at LB collection	Plasma volume for isolation (mL)	cfDNA yield (ng/ul)
1	Adenocarcinoma of pancreas	Primary diagnosis, early stages of treatment	8	4.36
5	Adenocarcinoma of sigmoid colon	Progressive disease, on treatment	8	9.24
11	ALK-positive non-small cell lung carcinoma	Progressive disease, on treatment	8	1.99
2	Cholangiocarcinoma of biliary tract	Primary diagnosis, early stages of treatment	9	12.20
3	Malignant tumor of unknown origin	Primary diagnosis, early stages of treatment	9	4.42
9	Triple-negative breast cancer	Progressive disease, on treatment	9	5.94
4	Adenocarcinoma of cecum	Progressive disease, on treatment	10	2.82
8	Adenocarcinoma of pancreas	Primary diagnosis, early stages of treatment	10	0.99
10	Intrahepatic bile duct carcinoma	Progressive disease, on treatment	10	5.50
6	Sarcoma	Primary diagnosis, early stages of treatment	12	13.9
7	Adenocarcinoma of pancreas	Progressive disease, on treatment	12	8.04

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Low ctDNA Abundance In specific Scenarios

- Even more pronounced with other body fluids potentially relevant for LB testing, like CSF, where there is also limited volume availability

Patient no.	Diagnosis	Disease course at LB collection	CSF volume for isolation (mL)	cfDNA yield (ng/ul)
CSF-1	Diffuse midline glioma, H3 K27-altered	Pre-surgery and pre-treatment	4	0.21
CSF-2	Diffuse midline glioma, H3 K27-altered	<1 month post-surgery, pre-treatment	4	NQ (<0.2 ng/ul)
CSF-3	Atypical teratoid/rhabdoid tumor	<1 month post-surgery, pre-treatment	4	0.26
CSF-4	Astrocytoma, IDH-mutant	<1 month post-surgery, pre-treatment	4	0.67
CSF-5	Infantile hemispheric glioma	Primary tumor, on treatment	4	NQ (<0.2 ng/ul)
CSF-6	Diffuse pediatric-type high-grade glioma, H3 and IDH-wildtype	<1 month post-surgery, pre-treatment	4	0.65
CSF-7	Diffuse midline glioma, H3 K27-altered	<1 month post-surgery, pre-treatment	4	7.58
CSF-8	Diffuse leptomeningeal glioneuronal tumor	Pre-surgery and pre-treatment	4	0.19
CSF-9	Diffuse midline glioma, H3 K27-altered	Disease progression, on treatment	4	NQ (<0.2 ng/ul)
CSF-10	Ependymoma	Relapse	6	NQ (<0.2 ng/ul)
CSF-11	Diffuse midline glioma, H3 K27-altered	Primary tumor, on treatment	6.5	NQ (<0.2 ng/ul)
CSF-12	Pineoblastoma	Disease progression, on treatment	30	2.40

NQ = not quantifiable by Qubit dsDNA HS assay with 1 ul sample input

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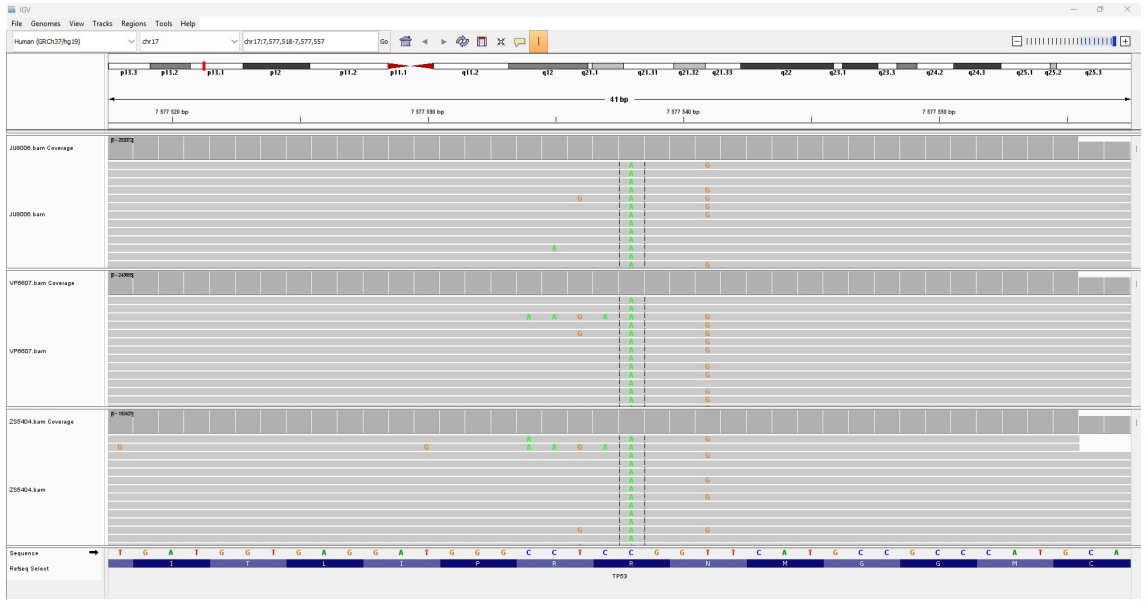
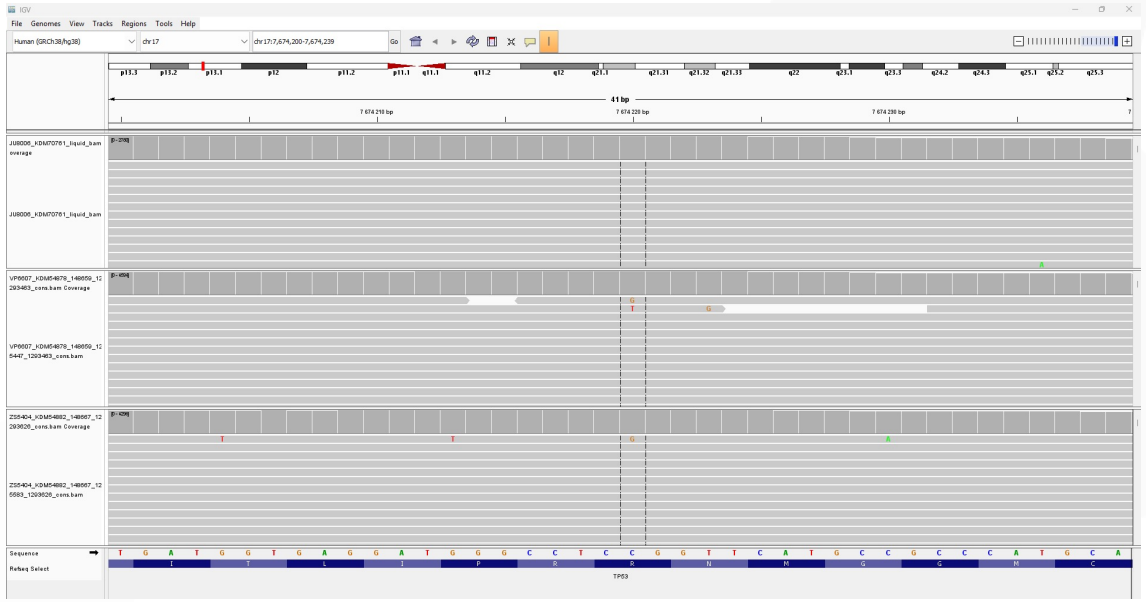
Assay Sensitivity Threshold

- VAF threshold to balance sensitivity x filter out technical noise
- Technology/sequencing platform-specific artifacts



TP53 p.Arg248Leu variant was called in several libraries prepared with kit by another manufacturer, emerged as an artifact



The same set of samples prepared with the OncoSELECT kit

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Biological background signals affecting specificity

- Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Patient no.	Gene	Transcript	HGVSc	HGVSp	Plasma VAF (%)
1	*ARID1A*	NM_006015.6	c.1440_1446delinsTTGCAACAA	p.(Q480Hfs*140)	0.21
1	*CHEK1*	NM_001274.5	c.575G>A	p.(W192*)	0.73
2	*CHEK2*	NM_007194.4	c.593-2A>G	p.?	0.54
2	*TP53*	NM_000546.6	c.722C>A	p.(S241Y)	0.37
2	*TP53*	NM_000546.6	c.584T>C	p.(I195T)	0.36
5	*GNAS*	NM_080425.4	c.2531G>A	p.(R844H)	0.29
9	*IDH2*	NM_002168.4	c.419G>A	p.(R140Q)	3.80

IGV-verified potentially relevant variant calls identified in OncoSELECT data (confirmed by other NGS solutions when possible)

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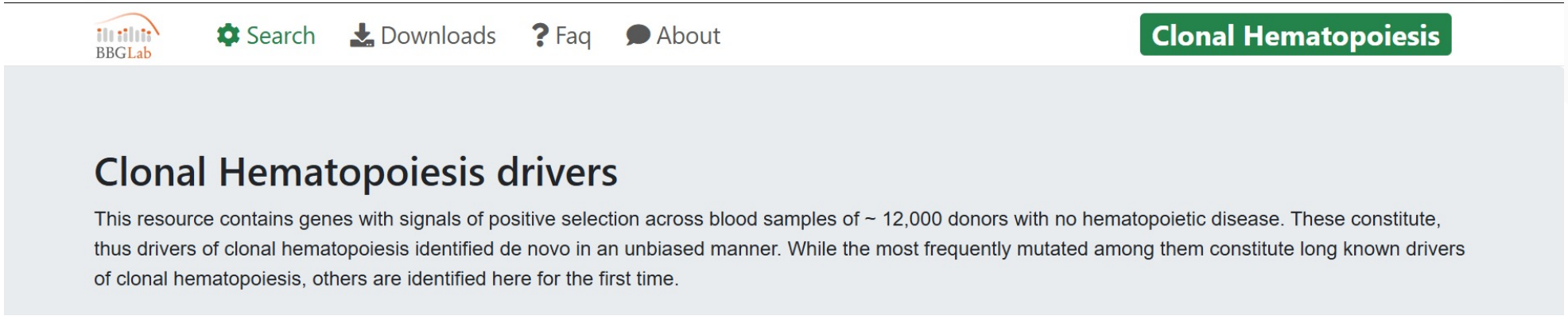

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Challenges Of Funding For Liquid Biopsy

- **Clinical utility threshold**
  - Expected impact on management and outcomes
- **Evidence gaps by indication**
  - Utility across tumor types
- **Unclear testing frequency during follow-up**
  - Intervals, duration – significant budget impact
- **Need for pathway standardization**
  - When to test, how to interpret, what action follows
- **Lack of harmonisation**
  - Different targets, analytes, sensitivity – complicated comparability

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Reimbursement Situation In The Czech Republic

- *EGFR, BRAF, KRAS,* and *NRAS* testing done routinely by qPCR

- Recognized indications for NSCLC, CRC, and malignant melanoma:
  - Primary diagnosis – in case tissue is not available
  - Relapse/disease progression – when there is a suspected change in the mutational spectrum of the tumor with potential therapeutic implications, and tissue biopsy is not feasible

- Separate reimbursement codes for the respective genes – the same code applied as in the case of tissue testing

- Not sufficient to cover NGS-based liquid biopsy testing

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Future Prospects For Liquid Biopsy Testing In The Clinic

- Standardization enabling scalable adoption and reimbursement
- Clear indication-specific placement in care pathways
- Prospective evidence linking liquid biopsy results to management decisions and outcomes
- Understanding and managing biological and technical limitations
- Potentially complimenting solid tissue testing through providing earlier testing in the pathway and easier follow-up

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Conclusion

- Liquid biopsy has the potential to significantly enhance precision oncology by providing minimally invasive and repeatable access to tumor-derived genomic information across the disease course
- While the outlook for liquid biopsy is promising, solid tissue testing remains essential and continues to represent the gold standard
- Tissue and liquid biopsy provide complementary insights, with their relative added value depending on the clinical context
- Successful adoption of liquid biopsy in the clinics and reimbursement frameworks will be driven mainly by
  - Standardization and harmonization
  - Clear clinical indications
  - Prospective evidence of clinical utility

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Key Take Aways For Our Audience

- Take care to formulate a testing strategy that draws upon both solid and liquid testing
- Create enough capacity to test in the laboratory to respond to demand from the clinic in a timely manner
- Draft a plan which includes your testing targets (goals, diseases and genes)
- Construct your validation plan and document the outcomes required
- Consider changes in context overtime (clinical requirements, assays, automation, staffing and logistics)
- Document routes to reimbursement as they are evolving all the time!

Key Take Aways For Our Audience

- Liquid Biopsy V Tissue NGS testing in Oncology:

- We believe they are both useful and provide an opportunity for a complementary strategy!

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Liquid Biopsy v Tissue NGS testing in Oncology: Competing Option or a Complementary Strategy?

Questions?

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